DESIGN AND SYNTHESIS OF DIVERSITY-ORIENTED PYRIMIDINE HETEROCYCLES AS ANTICANCER AGENTS

A

RESEARCH PROPOSAL

SUBMITTED

IN PARTIAL FULFILLMENT TO THE REQUIREMENT

FOR

DOCTOR OF PHILOSOPHY

IN

FACULTY OF PHARMACY

DISCIPLINE: PHARMACEUTICAL CHEMISTRY

By

Ms Shelly Pathania
(Enrollment No. - 17201FPE03)

MAHARAJA RANJIT SINGH PUNJAB TECHNICAL UNIVERSITY

BATHINDA (INDIA)

(March, 2020)

Contents

1. Title
2. Introduction
3. Literature Review
4. Research Issues
5. Problem Formulation
6. Research Objectives
7. Need of Proposed Work
8. Methodology
9. Bibliography
10. Place of Work
11. Resources Required
12. Extent of external assistance

1. **PROPOSED TITLE:** DESIGN AND DEVELOPMENT OF DIVERSITY-ORIENTED PYRIMIDINE HETEROCYCLES AS ANTI-CANCER AGENTS
2. **REVISED TITLE:** DESIGN AND SYNTHESIS OF DIVERSITY-ORIENTED PYRIMIDINE HETEROCYCLES AS ANTI-CANCER AGENTS
3. **INTRODUCTION**

2.1. CANCER

Cancer is one of the prevalent and high mortality rate diseases which involve uncontrolled cell proliferation. Approximately 1,735,350 new cases of cancer and 609,640 cancer deaths have been reported in 2018 in the United States. Amongst different types of cancer, breast cancer forms the second leading cause of death around the globe and has striking impact on women's health. Additionally, about 63,960 cases of in situ breast cancer have been diagnosed in women [1]. Every 1 in 33 women have chances of developing breast cancer through her lifetime. Smoking, alcohol intake, chemicals like asbestos, obesity are some of the risk factors which lead to breast cancer [2]. Breast cancer is categorized into five different subtypes, luminal A/B, HER2-positive (HER+), basal-like, claudin-low, and normal breast like, based on the expression levels of estrogen and progesterone receptors (ER and PR 80% of all breast cancers), HER2 (20% of breast cancers), cytokeratins 5/6, and claudins 3/4/7 [3]. The over expression of epidermal growth factor receptor (EGFR, ERBB1) has been reported with frequency ranging from 7 to 43% in breast cancer. The interaction between EGFR and HER2 are linked with poor survival of patients with HER2 positive breast cancer and showed resistance to trastuzumab, a monoclonal antibody approved drug for the treatment of breast cancer.[4]. The ERBB receptor on ligand binding undergoes homo or heterodimerization which result in the phosphorylation of tyrosine kinase domains in the cytoplasm and activate intracellular signalling such as PI3K /Akt, CDK (Cyclin dependent kinase) and mitogenic pathways *via* Ras/Raf/MAP kinase, lead to cell proliferation and survival. Accordingly, there are several chemotherapeutic agents, targeted at these molecular targets, available for breast cancer. However, one form of breast cancer, triple negative breast cancer which is characterized by the absence of molecular targets such as HER2, is resistant towards available FDA-approved chemotherapeutic agents. Several studies in literature have reported the relevance of CDK inhibition (especially CDK4 and CDK6) [5] and MAPK inhibition in

both estrogen receptor positive and triple negative breast cancer. Literature validate the fact that inhibition of CDK4/6 have emerged as useful target for the treatment of breast cancer with the discovery of potent, highly selective and FDA approved CDK4/6 inhibitors i.e. Palbociclib, Ribociclib and Abemaciclib, effective in both estrogen receptor-positive (ER+) and HER2 negative advanced metastatic breast cancer [6].

On the other hand, inhibition of MAPK (mitogen activated protein kinase), responsible for the inhibition of ERK1/2 pathway, is another important target involved in the treatment of both estrogen receptor positive and triple negative breast cancer. Two FDA approved MAPK inhibitor i.e. trametinib and cobimetinib have been reported for the treatment of patients suffering with V600E mutated metastatic melanoma [7], which provide an idea for further designing of MAPK inhibitors. Overall many ERK inhibitors have been reported for the management of various resistant and non-resistant cancers by inhibiting the ERK1/2 pathway. Furthermore, in literature it has been reported that a correlation exist between MAPK signalling pathway and CDK signalling pathway in controlling cell cycle progression. The inhibition of CDK activity has a direct control over the activity of MAPK and vice versa [8].

So considering these parameters, we envisaged designing of novel pyrimidine based small molecule inhibitors for molecular targets of MAPK signalling pathway to provide an effective and attractive option for targeted therapy in breast cancer [9, 10].

2.2. PYRIMIDINE DERIVATIVES

Pyrimidine and its fused derivatives, due to being an inextricable part of DNA and RNA, possess essential role in numerous biological processes and are established as a medicinally significant scaffold. Pyrimidine-condensed derivatives exhibit broad spectrum of biological activities such as antitubercular, antibacterial, antifungal, antiviral, anti-inflammatory, antimalarial, anticancer, antidiabetic and anti-HIV. Several retrosynthetic approaches are available for the synthesis of pyrimidine-fused analogues which offers enormous scope in the field of medicinal chemistry. Ring fused pyrimidine and their innumerable derivatives continue to hold the attention of medicinal chemists since their presence in the biologically active resources have been known to prompt additive effects on the efficacy of the molecules. Thus, in our proposed work we intend to perform diversity-oriented synthesis of pyrimidine derivatives as anticancer agents.

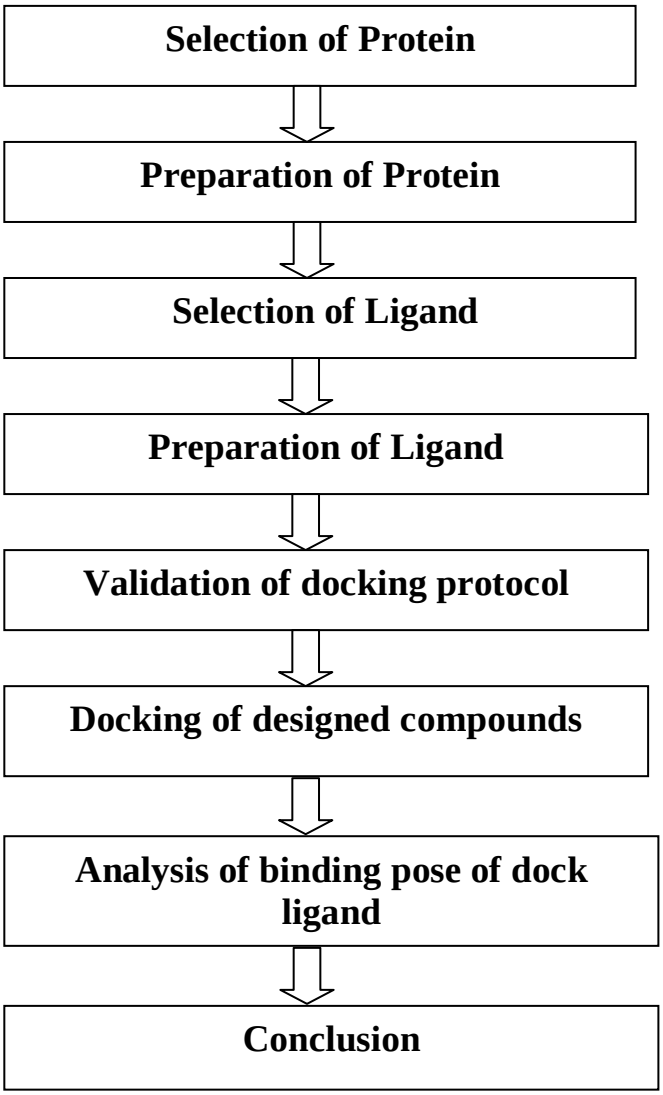
2.3 DESIGN CONSIDERATIONS FOR PROPOSED SCAFFOLD

Drug design is a rational application for the identification of lead and its modifications to improve its activity and drug-like properties for a pathophysiological target under investigation. The selection of proposed research work has been done on the basis of some pre-design considerations by thorough literature survey. It has been found in literature that various pyrimidine derivatives possess anticancer activity by binding to a variety of targets. Some of them include EGFR (Epidermal growth factor receptor), FLT3 (fms like tyrosine kinase), HER2 (Human epidermal growth factor receptor 2), BRAF (B – Rapidly Accelarated Fibrosarcoma Kinase), c-MET (Hepatocyte growth factor receptor), MAPK (Mitogen activated protein kinase), FGFR 1/2/3 (Fibroblast growth factor receptor), CDK 4/6 (Cyclin-dependent kinase), etc. These molecular targets are reported to provide binding sites for the various pyrimidine derivatives for the treatment of different types of cancers.

Further, designing of the molecules will be done considering the catalytic domain of selected targets from MAPK signalling pathway. Various *in-silico* approaches such as structure based drug designing and ligand based drug designing, would be utilized in the holistic designing of the molecules.

2.4 Docking studies

It is a well-established computational technique which predicts the binding energy and pose of a ligand in the binding pocket of a target protein between two molecules. It also finds out the energy minimized conformation and orientation of ligand which would form a complex with target protein. The ligand usually fits within protein's cavity which is determined by search algorithm. When protein cavities come in contact with any external compounds, they become active and thus also known as active sites. For successful docking, conformational changes are necessary in the protein and the ligand. Various softwares (Autodock, Chimera, MOE, Accelrys Discovery studio, Schrodinger Maestro) are available in market that helps to predict the binding mode between the protein and ligand. Thus, molecular docking emerged as an important tool in discovery of new lead molecules for a particular pathophysiological target. Molecular docking procedure proceeds through following steps:



4. LITERATURE REVIEW

3.1. Substituted pyrimidine derivatives as CDK inhibitor

The purine ring system (fusion of pyrimidine and imidazole) is used in a number of CDK inhibitors. The various derivatives reported are olomoucine **1**, purvalanol B **2**, roscovitine **3**, bohemine **4**. All these compound exhibit CDK inhibitory activity. Olomoucine showed modest potency for CDK1/cyclin B with IC_{50} = 7 µM, for CDK2/cyclin E with IC_{50} = 7 µM and CDK5/p35 with IC_{50} value of 3 µM. Purvalanol B also exhibit against CDK2/cyclin A inhibitory activity with IC_{50} of 6 nM. This compound also shows selectivity towards CDK4/cyclin D1 and to other kinases [11].

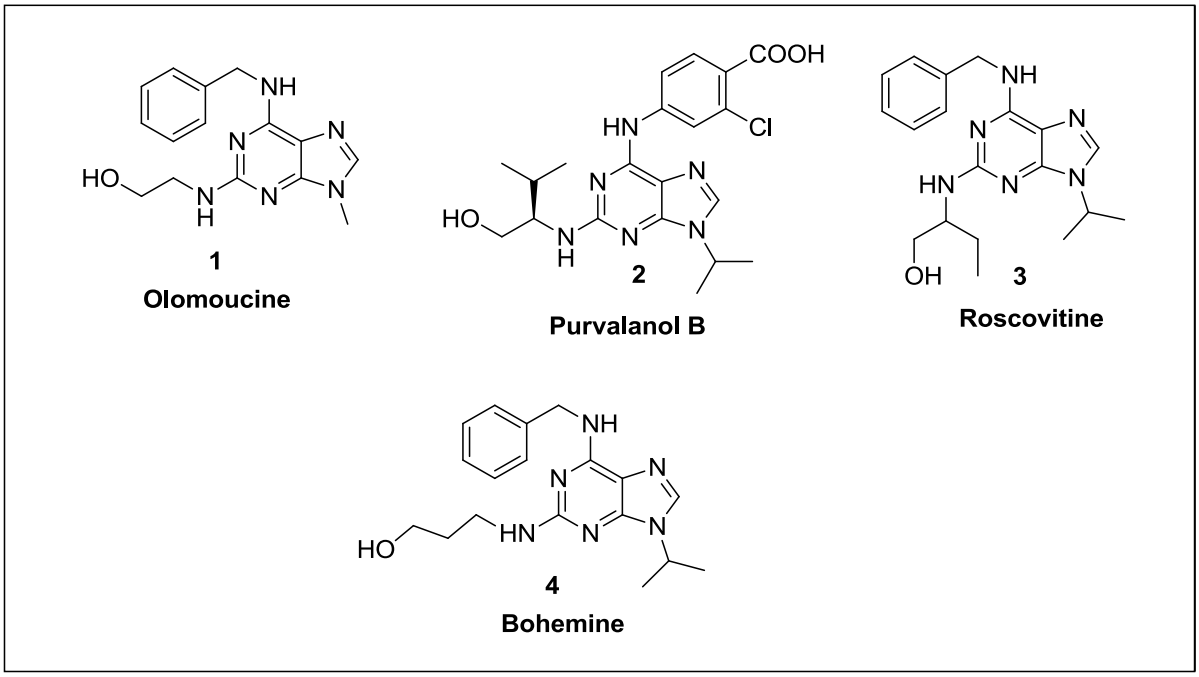


Fig. 1- Various substituted pyrimidine derivatives as CDK inhibitor

Beattie et al. reported 4, 6-bis anilino pyrimidines **5** as inhibitors of the cyclin dependent kinase, CDK4 *via* high throughput screening. The inhibitor showed CDK4 inhibitory activity with an IC_{50} value = 15µM and also exhibit some selectivity against CDK2 inhibitory activity with IC_{50} =93 µM. The structure consists of two rings A and B, on which modifications were done. It has been observed that a modification in ring B increases in potency with 10 fold [12].

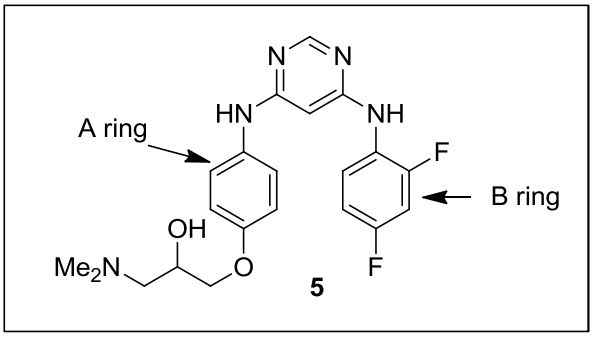


Fig. 2- 4, 6-bis anilino pyrimidines

Wang et al reported a novel series of 4-(2,3-dihydro-1*H*-benzo[*d*]pyrrolo[1,2-*a*]imidazole-7-yl)-*N*-(5-(piperazin-1-ylmethyl)pyridine-2-yl)pyrimidin-2-amine derivatives as potent CDK 4/6 inhibitors based on Abemaciclib (FDA approved CDK4/6 inhibitor). The compounds

were evaluated for CDK inhibitory activity, and it has been found that compound **6** exhibits good CDK4 and CDK6 inhibitory activity with IC_{50} value of 7.4 and 0.9 nM, respectively and also exhibit good pharmacokinetic properties [13].

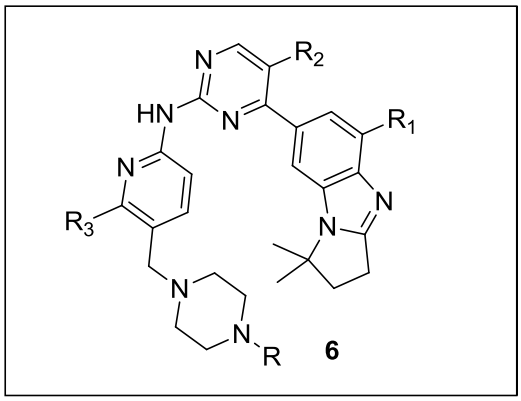


Fig. 3- Substituted pyrimidine derivatives as CDK4/6 inhibitors

3.2. Substituted pyrimidine derivatives as MAPK inhibitor

Dong et al reported the series of 5-phenylamino-8-methylpyrido[2,3-*d*]pyrimidine-4,7(3*H*,8*H*)-dione as MEK inhibitor. The compounds were developed *via* structure based drug designing. All the synthesized compounds were evaluated against MEK1 inhibitory activity, and from results, a molecule named as TAK-733 **7** was found to active in enzymatic activity against MEK enzyme with IC_{50} = 3.2 nM and an EC_{50} of 1.9 nM against ERK phosphorylation in cells. Docking studies revealed that the oxygen of pyridone forms a hydrogen bond with the backbone NH of Ser212 whereas pyrimidinone forms hydrogen bond with residue Lys97. The fluoro and iodo substituted aniline moiety fit well in the lipophilic pocket [14].

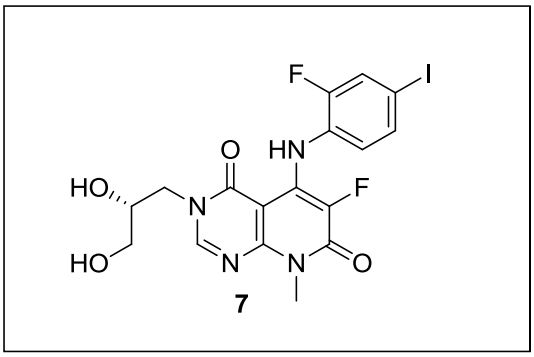


Fig. 4 - TAK-733 as MAPK inhibitor

Seerden et al reported the derivatives of pyrimidine fused with imidazole ring as selective p38α MAPK inhibitor. The compound **8** exhibit good MAPK inhibitory activity with IC_{50} value of 250 nM in comparison to reference compound JNJ7583979, which is a potent and selective p38α MAPK inhibitor with IC_{50} value = 8.5 nM. The existence of the lipophilic substituents like (*S*)-1-phenylethylamino group in JNJ7583979 is important for the high potency of the compound. Similarily, the presence of triazolyl acetate group in compound **8** increases the aqueous solubility by 11 fold and 2-fold decrease in the p38α MAPK inhibition [15].

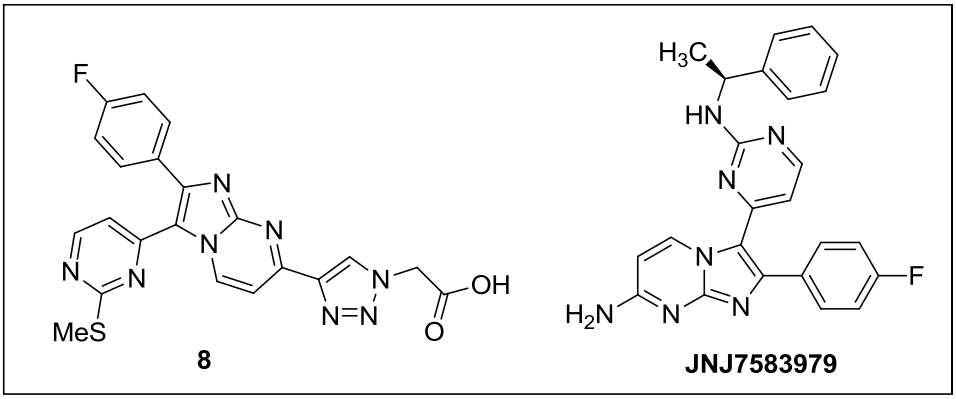


Fig. 5 - Imidazopyrimidine derivatives as p38α MAPK inhibitor

5. RESEARCH ISSUES/GAPS

MAPK pathway is one of the best-characterized signalling cascades that regulates a variety of cellular functions and dysregulation in it leads to occurrence and progression of cancers. MAPK pathway is composed of RAS, RAF, MEK and ERK members. RAS has been identified as an oncogene and is activated in approximately one-third of all cancers. However, due smooth and floppy nature of the tertiary structure of RAS, it hardly provides a binding pocket for small molecule inhibitors and thus is hard to target. Moreover, oncogenic mutant RAS are insensitive to GTP hydrolysis, resulting in constitutively active protein & the affinity of RAS for GTP is extraordinarily high, and it is almost impossible to develop a competitive binding strategy as a result.

Similarly, BRAF mutations have been widely identified in tumours, with a significant percentage of all human cancers. And although BRAF inhibitors have achieved clinical benefits, the extent and duration of treatment with BRAF inhibitors is variable and the extremely high frequency of emergence of drug resistance eventually leads to failure of the

treatment. With the emergence of problems with RAS and RAF inhibitors, targeting MEK1/2 attracted attention.

However, as with other small molecule inhibitors, patients treated with MEK inhibitors also develop drug resistance within several months.

6. RESEARCH PROBLEM/PROBLEM FORMULATION

Compared to RAF and MEK inhibitors, the development of ERK1/2 selective inhibitors lags far behind. However, with the difficulties faced by RAF and MEK inhibitors, discovery and development of ERK inhibitors has gained an increasing interest.

ERK sits at a unique position in the MAPK pathway, and is the only activator that is able to stimulate the wide variety downstream substrates. ERK1/2 inhibitors can reverse the abnormal activation of MAPK pathway induced by upstream mutations and also can overcome the acquired drug resistance induced by upstream kinases inhibitors. Moreover, crosstalk between ERK and other pathways has been shown to be crucial for determining cell fate.

7. RESEARCH OBJECTIVES

- To design pyrimidine containing derivatives using molecular modelling tools.
- To synthesize the designed compounds.
- To characterize the synthesized molecules using spectral techniques.
- To evaluate *in vitro* anti-proliferative potential of the synthesized compounds via enzymatic assays and cancer cell lines (MCF-7, MDA-MB-231 and A549).

7. NEED OF PROPOSED RESEARCH

We hypothesized to design and develop pyrimidine-based molecules targeting ERK1/2, to manage various types of MAPK-dependent cancer. The designed molecules are needed to overcome the issues faced by previously developed molecules for multiple members of MAPK pathway such as RAS, BRAF and MEK1/2.

Special focus during the in-silico analysis will be given to keep the pyrimidine based hits, considering medicinal importance of the scaffold.

8. METHODOLOGY ADOPTED

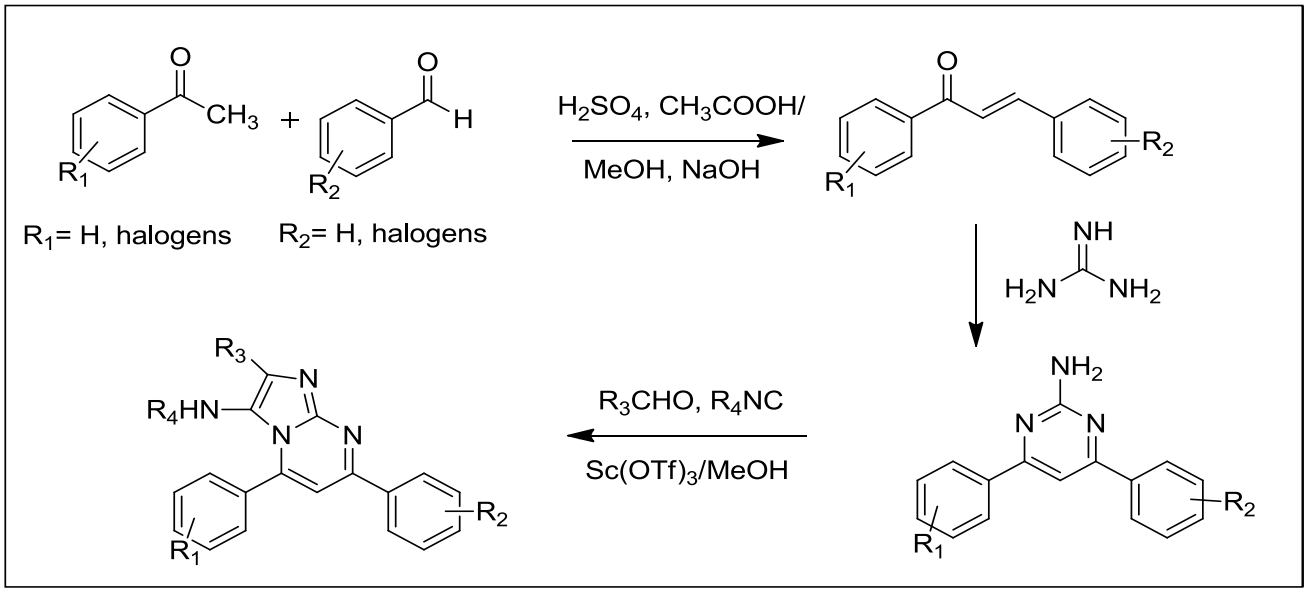
8.1 Molecular modelling:

Various molecular strategies will be utilized to design pyrimidine based small molecule heterocycles. Structure based drug designing techniques namely, molecular docking analysis, molecular dynamic simulations etc., will be utilized to identify the binding mode and affinity of the designed molecules. Information available about already available inhibitors will also be incorporated to compensate for the ligand based drug designing approach. Other strategies such as scaffold hopping and fragment based drug designing can also be utilized to improve the putative potency of the designed molecules.

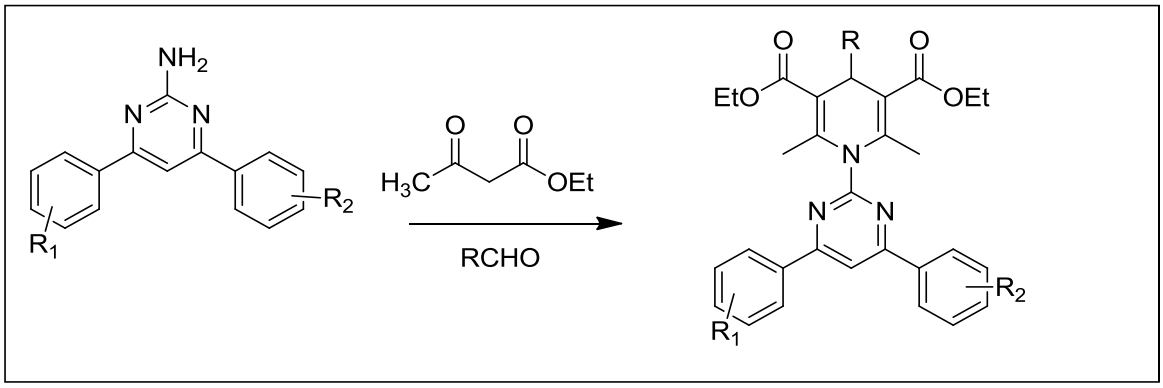
8.2 Chemical synthesis

Designed pyrimidine based molecules will be synthesized utilizing well-documented synthetic protocols, as given below, but not limited to.

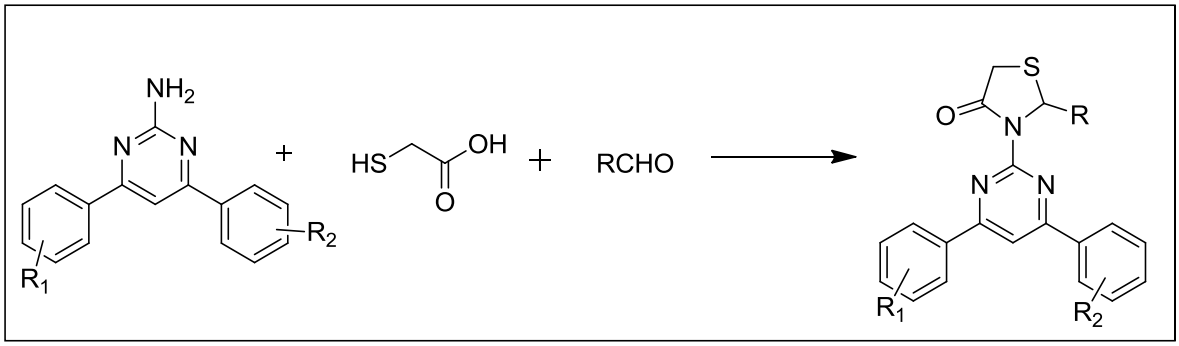
Scheme 1:



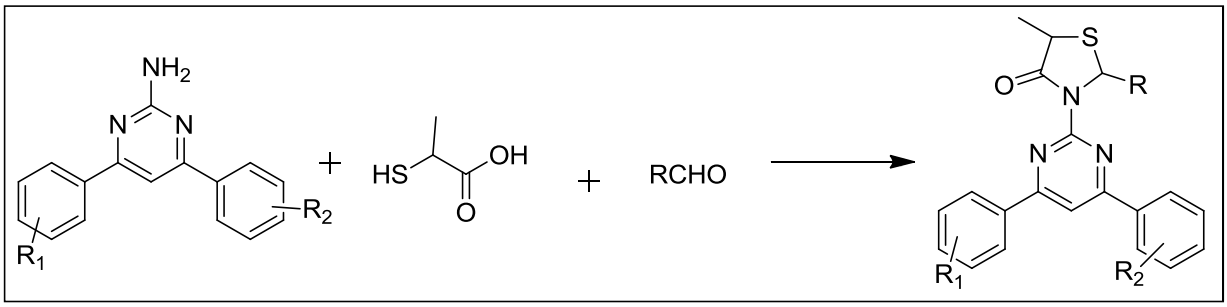
Scheme 2:



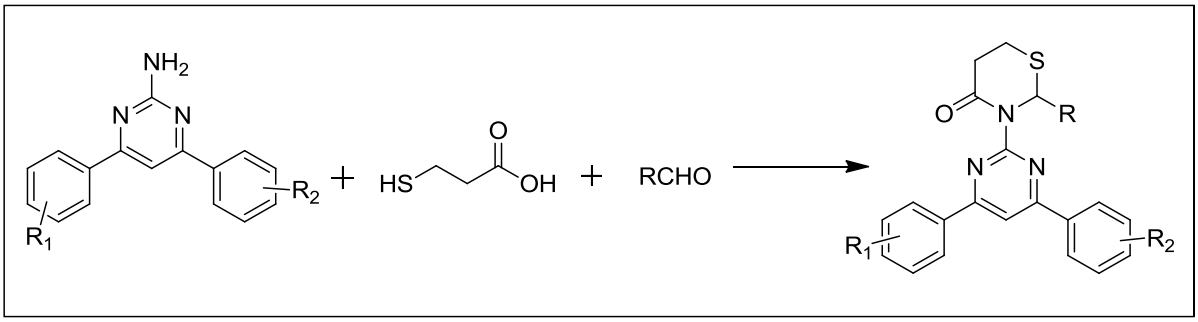
Scheme 3:



Scheme 4:



Scheme 5:



8.3 Biological evaluation

The synthesized compounds will be evaluated for their anti-proliferative potential using in-vitro biological assay methods, both enzymatic and cell based assays. The cell lines used in this study will be MCF-7, MDA-MB-231and A549.

BIBLIOGRAPHY

1. R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2018, *CA: a cancer journal for clinicians* 68 (2018) 7-30.

2. A.E. Heitz, R.N. Baumgartner, K.B. Baumgartner, S.D. Boone, Healthy lifestyle impact on breast cancer-specific and all-cause mortality, *Breast cancer research and treatment* 167 (2018) 171-181.

3. J.L. Hsu, M.-C. Hung, The role of HER2, EGFR, and other receptor tyrosine kinases in breast cancer, *Cancer and Metastasis Reviews* 35 (2016) 575-588.

4. E. Provenzano, G.A. Ulaner, S.-F. Chin, Molecular Classification of Breast Cancer, *PET Clinics* (2018).

5. E.L. Mayer, Targeting breast cancer with CDK inhibitors, *Current oncology reports* 17 (2015) 20.

6. B. Laderian, T. Fojo. *CDK4/6 Inhibition as a therapeutic strategy in breast cancer: palbociclib, ribociclib, and abemaciclib*. in *Seminars in oncology*. 2017: Elsevier.

7. Y. Cheng, H. Tian, Current development status of MEK inhibitors, *Molecules* 22 (2017) 1551.

8. E. Durandau, S. Pelet, Cross-regulation between CDK and MAPK control cellular fate, *bioRxiv* (2018) 274787.

9. H. Lee, A. Seo, E. Kim, M. Jang, Y. Kim, J. Kim, S. Kim, H. Ryu, I. Park, S. Im, Prognostic and predictive values of EGFR overexpression and EGFR copy number alteration in HER2-positive breast cancer, *British journal of cancer* 112 (2015) 103.

10. B. O'leary, R.S. Finn, N.C. Turner, Treating cancer with selective CDK4/6 inhibitors, *Nature reviews Clinical oncology* 13 (2016) 417.

11. T.M. Sielecki, J.F. Boylan, P.A. Benfield, G.L. Trainor, Cyclin-dependent kinase inhibitors: useful targets in cell cycle regulation, *Journal of medicinal chemistry* 43 (2000) 1-18.

12. J.F. Beattie, G.A. Breault, R.P. Ellston, S. Green, P.J. Jewsbury, C.J. Midgley, R.T. Naven, C.A. Minshull, R.A. Pauptit, J.A. Tucker, Cyclin-dependent kinase 4 inhibitors as a treatment for cancer. Part 1: identification and optimisation of substituted 4, 6-bis anilino pyrimidines, *Bioorganic & medicinal chemistry letters* 13 (2003) 2955-2960.

13. Y. Wang, W.-J. Liu, L. Yin, H. Li, Z.-H. Chen, D.-X. Zhu, X.-Q. Song, Z.-Z. Cheng, P. Song, Z. Wang, Design and synthesis of 4-(2, 3-dihydro-1H-benzo [d] pyrrolo [1, 2-a] imidazol-7-yl)-N-(5-(piperazin-1-ylmethyl) pyridine-2-yl) pyrimidin-2-amine as a highly potent and selective cyclin-dependent kinases 4 and 6 inhibitors and the discovery of structure-activity relationships, *Bioorganic & medicinal chemistry letters* 28 (2018) 974-978.

14. Q. Dong, D.R. Dougan, X. Gong, P. Halkowycz, B. Jin, T. Kanouni, S.M. O'Connell, N. Scorah, L. Shi, M.B. Wallace, Discovery of TAK-733, a potent and selective MEK allosteric site inhibitor for the treatment of cancer, *Bioorganic & medicinal chemistry letters* 21 (2011) 1315-1319.

15. J.-P.G. Seerden, G. Leusink-Ionescu, T. Woudenberg-Vrenken, B. Dros, G. Molema, J.A. Kamps, R.M. Kellogg, Synthesis and structure–activity relationships of 4-fluorophenyl-imidazole p38α MAPK, CK1δ and JAK2 kinase inhibitors, *Bioorganic & medicinal chemistry letters* 24 (2014) 3412-3418.

PLACE OF WORK: Department of Pharmaceutical Chemistry, ISF College of Pharmacy, Moga, Punjab.

9. RESOURCES/SOFTWARES REQUIRED:

- Fume Hood (Available)
- Rota-evaporator (Available)
- Columns (Available)
- Glasswares (Available)
- Microwave (Available)
- HPLC (Available)
- UV (Available)
- FTIR (Available)
- NMR (Not available)
- Mass Spectrometry (Not available)
- AutoDock (Available)
- GOLD (Available)
- MOE (Available)

10. EXTENT OF EXTERNAL ASSISTANCE REQUIRED: Required for NMR and Mass spectrometry data (Payment basis).

Candidate	**Co-Supervisor**	**Supervisor**
(Shelly Pathania)	**(Dr. R.K. Rawal)**	**(Dr R. K. Narang)**